

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

Claims 1-2 (canceled)

3. (currently amended) An isolated conjugate comprising

- (a) at least
 - (i) one ubiquitin or
 - (ii) ubiquitin fused with another molecule, which is employed for purification or visualization; and
- (b) a protein selected from the group consisting of aprataxin, synaptogamin-like protein (SLP), high mobility group protein 17 (HMG17), PIN2-interacting protein 1 (PinX1), CBF1 interacting corepressor (CIR), high mobility group nucleosomal binding protein 3 (HMGN3), hematopoietic stem/progenitor cell 144 (HSPC144), Cullin 3, cell division cycle 6 (CDC6), and fragments and variants thereof;

wherein (i) said variants comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins and (ii) said fragments comprise polypeptides of at least 50 amino acids having at least 90% identity to a sequence fragment of said protein; and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue and an internal Lys residue.

4. (previously presented) The conjugate of claim 3, wherein said conjugate is immobilized on a support and/or linked to a label.

5. (currently amended) A method for producing a conjugate comprising

- (a) at least one
 - (i) ubiquitin or
 - (ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and

- (b) a protein selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, tau, and fragments and variants thereof; comprising:
 - A) forming a mixture comprising a vector containing a clone coding for said protein, or said fragment or variant thereof ~~or derivative thereof~~, an in vitro transcription/translation system, an N-rule ubiquitylation system and, optionally, a proteasome inhibitor; and
 - B) incubating said mixture to allow production of said conjugate;
- wherein (i) said variants comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins and (ii) said fragments comprise polypeptides of at least 50 amino acids having at least 90% identity to a sequence fragment of said protein; and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue and an internal Lys residue.

6. (previously presented) The method of claim 5, further comprising:

- C) isolating said conjugate.

7. (original) The method of claim 6, wherein said isolating is done by binding to an antibody specific to a poly-ubiquitin chain.

8. (original) The method of claim 6, wherein said isolating is done by binding to an antibody specific for said protein.

Claims 9-16 (canceled)

17. (currently amended) A composition comprising a conjugate comprised of

- (a) at least one
 - (i) ubiquitin or

- (ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and
- (b) a protein selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, and fragments and variants thereof; wherein (i) said variants comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins and (ii) said fragments comprise polypeptides of at least 50 amino acids having at least 90% identity to a sequence fragment of said protein; said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue plus an internal Lys residue, and said conjugate is immobilized on a support and/or linked to a label.

Claims 18-56 (canceled)

57. (previously presented) An isolated conjugate comprising

- (a) at least one
 - (i) ubiquitin or
 - (ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and
 - (b) a recombinant protein selected from the group consisting of tau, and fragments and variants thereof;
- wherein (i) said variants comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins and (ii) said fragments comprise polypeptides of at least 50 amino acids having at least 90% identity to a sequence fragment of said protein; and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue plus an internal Lys residue.

58. (previously presented) The conjugate of claim 57, wherein said conjugate is immobilized on a support and/or linked to a label.

59. (previously presented) A composition comprising a conjugate comprised of

- (a) at least one
 - (i) ubiquitin or
 - (ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and
- (b) a protein selected from the group consisting of tau, and fragments and variants thereof;

wherein (i) said variants comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within tau and (ii) said fragments comprise polypeptides of at least 50 amino acids having at least 90% identity to a sequence fragment of said protein; and said conjugate is formed via N-end rule ubiquitylation of a polypeptide comprising a destabilizing N-terminal residue plus an internal Lys residue, and said conjugate is immobilized on a support and/or linked to a label.

60. (previously presented) The conjugate of claim 3, wherein said fragment is an activated fragment of a protein, said fragment having an exposed N-degron.

61. (previously presented) The conjugate of claim 60, wherein said activated fragment is immobilized on a support and/or linked to a label.

62. (previously presented) The conjugate of claim 57, wherein said fragment is an activated fragment of a protein, said fragment having an exposed N-degron.

63. (previously presented) The conjugate of claim 62, wherein said activated fragment is immobilized on a support and/or linked to a label.

64. (previously presented) The method of claim 6, wherein said isolating is done by binding to said molecule.

65. (previously presented) An isolated conjugate comprising

- (a) at least one
 - (i) ubiquitin or
 - (ii) ubiquitin derivatized with another molecule, which is employed for purification or visualization; and
- (b) a protein;

said conjugate made by a process comprising:

- A) forming a mixture comprising
 - (i) a vector containing a clone coding for said protein, wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, tau, and fragments and variants thereof; and (i) said variants comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins and (ii) said fragments comprise polypeptides of at least 50 amino acids having at least 90% identity to a sequence fragment of said protein; and said fragment or variant thereof ~~or derivative~~ comprises a destabilizing N-terminal residue and an internal Lys residue;
 - (ii) an in vitro transcription/translation system;
 - (iii) an N-rule ubiquitylation system; and
 - (iv) optionally, a proteasome inhibitor; and
- B) incubating said mixture to produce said conjugate.

66. (previously presented) The conjugate of claim 65, wherein said conjugate is immobilized on a support and/or linked to a label.